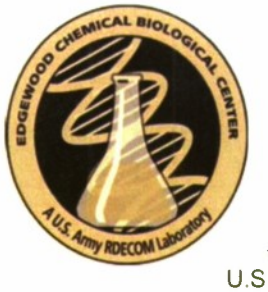

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ECBC-TR-750

CAPILLARY INLET CONCENTRATOR FOR THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS)

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14. ABSTRACT
The standard method for concentration of virus samples with the Integrated Virus Detection System (IVDS) is to use a tangential flow filtration system to reduce the volume of the sample, while removing impurities such as salts and small cellular debris. This report investigated the IVDS' ability to concentrate the virus sample as well as remove impurities with a filtration attachment on the inlet side of the electrospray module. The filtration attachment or concentrator allowed the virus sample to be purified and concentrated and then analyzed with the IVDS without removing the sample from the IVDS.

15. SUBJECT TERMS

Virus detection	Differential mobility analyzer	Condensation particle counter
Biological agents	Virus concentration	MS-2
Capillary	Virus filtration	Electrospray injection

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PREFACE

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CAPILLARY INLET CONCENTRATOR FOR THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS)

1. INTRODUCTION

The standard method for concentration of virus samples with the Integrated Virus Detection System (IVDS) is to use a tangential flow filtration system to reduce the volume of the sample, while removing impurities such as salts and small cellular debris. The investigators studied the ability to concentrate the virus sample as well as remove impurities with a filtration attachment on the inlet side of the electrospray module. The filtration attachment or concentrator allowed the virus sample to be purified, concentrated, and then analyzed with the IVDS without removing the sample from the IVDS.

1.1 Capillary Inlet Concentrator[1]

An adaptation was produced for the inlet sample holder for the electrospray module to allow additional concentration of viral samples before their injection into the electrospray capillary. The adaptation involved new machining of the sample holder to use the existing overpressure in the electrospray inlet to filter and concentrate samples. The newly machined module allowed the filtration of samples directly on the electrospray inlet with a commercially available wedge type centrifuge filter. The concentrator can eliminate the time-consuming centrifugation step, which can take up to 60 min, and allow direct analysis of the sample after its concentration. Tests were performed to determine concentration efficiency with a virus sample.

1.2 Concentrator Tests

A sample of MS2 bacteriophage, initial sample concentration of ~$1x10^5$ particles/mL, was placed in the wedge filter (100K Da) in the concentrator module. The sample (500 µL) was analyzed with the IVDS (Figure 1) and then concentrated to 100 µL and analyzed again (Figure 2) after a 4 min concentration. The scans show a 16 fold increase (initial counts in region of interest [ROI] = 531; final counts in ROI = 8674) in counts in the concentrated sample, measuring between 23.3 and 27.9 nm. In addition, during the concentration step, the wedge filter removed the large salt peak below 13 nm.

[1] Patent Pending, Dr. C. H. Wick

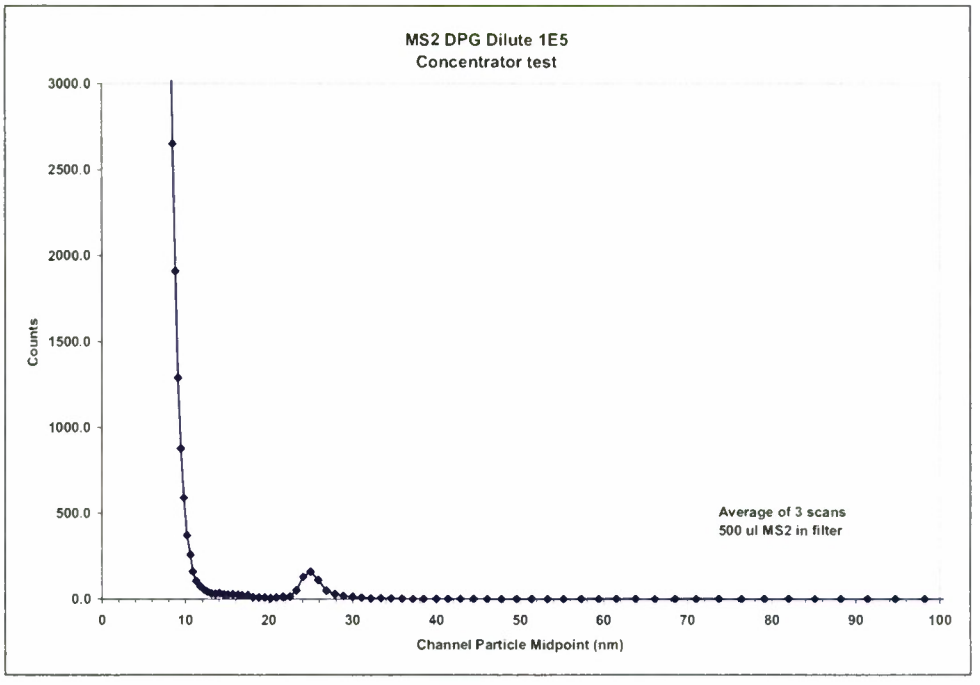

Figure 1. Initial MS2 Sample in Concentrator

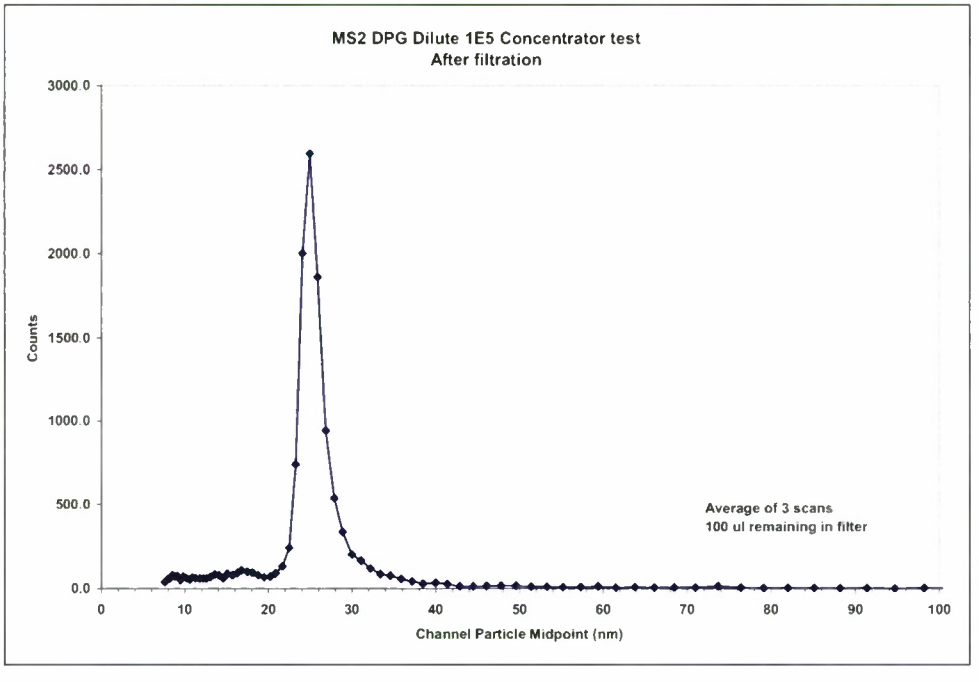

Figure 2. Final MS2 Sample in Concentrator

A second test with the concentrator was to determine its ability to perform partial concentrations with the assembly. The same stock sample of MS2 (500 µL) was placed in the wedge filter. The sample was filtered in 1 min increments and then analyzed with the IVDS.

The filtration was stopped when the sample reached a volume of 80 µL after 10 min. Again, the salt peak was removed after the first minute of filtration, and the subsequent scans were very clean below 15 nm as shown in Figure 3. The MS2 counts in the ROI (23.3-27.9 nm) increased with each increment of filtration and are listed in Table 1. A photograph and parts diagram of the concentrator are shown in Figure 4.

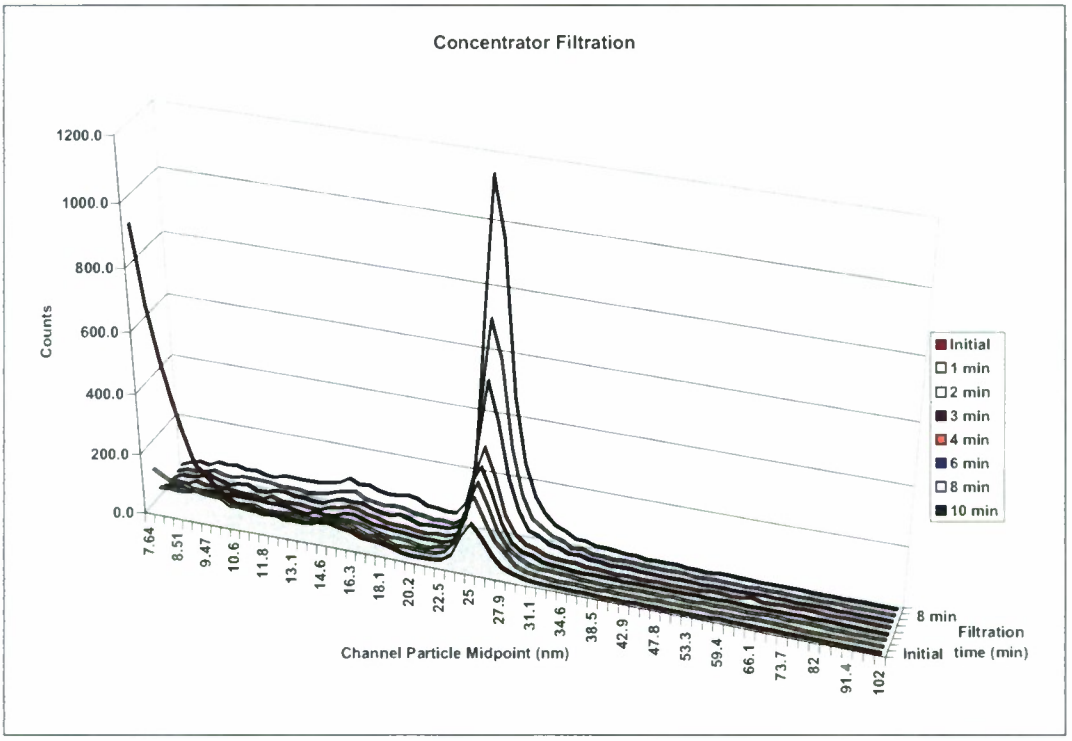

Figure 3. Concentrator IVDS Analysis in 1 min Increments

Table. MS2 Counts from Timed Concentrator Analyses

Time (min)	ROI Counts 23.3-27.9 nm
0	559
1	812
2	883
3	1023
4	1153
6	1793
8	2432
10	3744

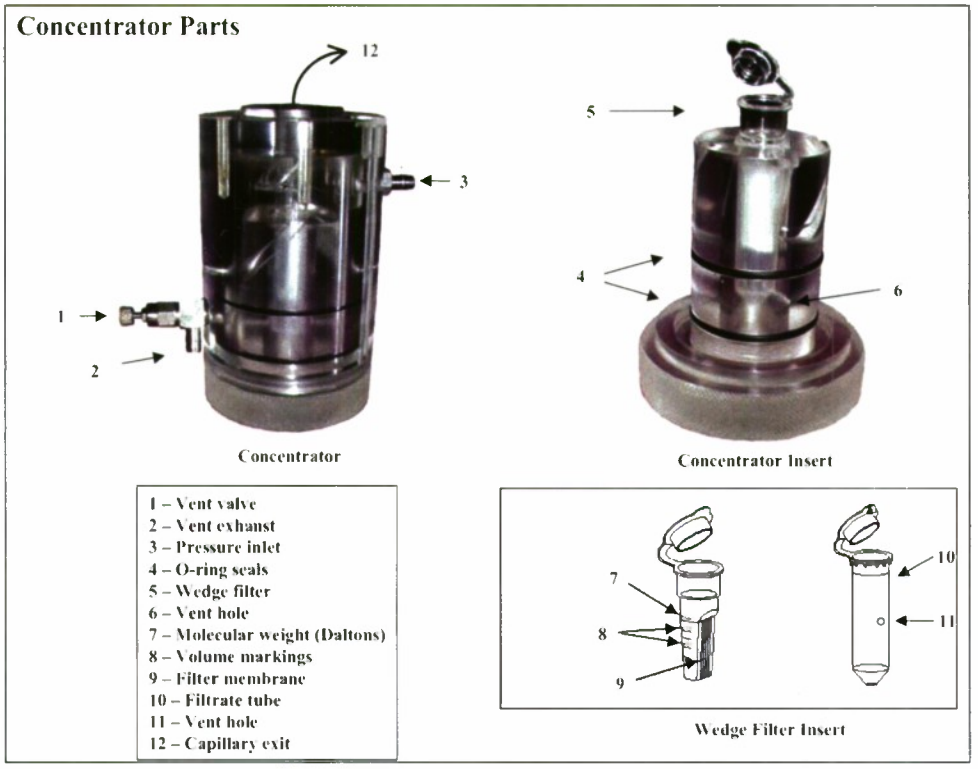

Figure 4. Concentrator and Filter View

2. CONCLUSION AND DISCUSSION

The concentrator assembly was able to increase the amount of counts and the concentration of a sample, while reducing the volume. An added benefit to the filter insert was the removal of salt material that can interfere, if present in sufficient quantity, with the Integrated Virus Detection System analysis of viruses.

This method is indicated for samples of low concentrations and where an additional concentration step will increase the particle count. This is particularly convenient for samples that contain unknown particle concentrations. The particles can be concentrated on the instrument and a new particle count determined without further manipulation.

This method has application for aerosol samples. In place of a liquid sample, an aerosol stream can be directed past the wedge filter insert to concentrate virus sized particles. This is adventitious for clean air streams with low concentrations of virus sized particles. After an appropriate period of time the regular method of placing a liquid in the sample tube is followed and a particle count determined using the standard procedures.